Bachelor or Master thesis BIOVIT 2021/22

Topic/Title (Norwegian): Virkningen av forskjellige lysregimer på fiskevelferd

Topic/Title (English): Impact of different light regimes on fish welfare

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Pictures:

From left to right: Romain Fontaine, Eirill Ager-Wick, Marco Vindas, Hege Lund, Ida Beitnes

Summary (Describe the topic/thesis, type of thesis work: field work, laboratory work, literature study) :

Abstract:
Light duration (day length) is used by animals to synchronize biological and physiological functions with the seasons. However, human activities are currently altering this synchronization for many animal species by modifying the light environment. Sometimes, this is done on purpose as for instance in aquaculture where continuous light is used to grow salmon faster. Other times, it is just a side effect of human activities. For instance, on the coast or in cities where artificial light at night (ALAN) in streets, produce light pollution. While continuous light is forbidden for most of other farmed animals, this is still commonly used in many fish farms. In addition, ALAN were shown to disturb the seasonal reproduction in a wide range of animal species, including mammals, insects, and fishes. Surprisingly, not much is known about the effect of these human-modified light regimes on fish welfare. In this project, we will use small model fish (Japanese medaka) to investigate the effects of different light regimes including continuous light and ALAN on fish welfare by investigating fish behavior, growth, reproduction, stress, and immune system.

Project background:
Light is the most stable environmental factor over the years with long days during summer and short days during winter. It has therefore been used by most of animals through evolution to synchronize biological and physiological functions (e.g. growth, reproduction) with the seasons. However, human activities are currently altering this synchronization for many animal species by modifying the light environment.

Indeed, several important Nature articles have recently brought to light the problem for animal life of light pollution in cities. This is a concern for wild as well as farmed animals living near the cities. In fish for instance, it has been shown that ALAN perturb seasonal reproduction along the coast. In addition, in the aquaculture industry, raising fish under continuous light is a common

protocol to delay puberty, allowing animals to grow faster. However, such treatment might have welfare issues for the animals. Indeed, continuous light treatment is forbidden for most of other farmed animal species.

In this project we propose to use the medaka, a teleost fish for which we have many tools to study its biology and physiology. Interestingly, medaka is a species with a seasonal reproduction. In laboratory conditions (14h of light), this species spawns daily. However, when reducing the light duration to 10h of light, it stops reproducing, making this species interesting to investigate the effect of light on seasonal reproduction. *We thus propose to investigate the effect of continuous light treatment and ALAN on several aspects of fish welfare including fish behavior, growth, reproduction, stress and immune system.*

Figure 1: Medaka (Oryzias latipes)

Hypotheses:

We hypothesize that non-natural light regimes (ALAN or continuous light) affect the fish welfare, especially:

1- **affect animal development, leading to malformation of the brain and heart.**
2- **affect sexual maturation and seasonal reproduction, leading to poor reproduction success.**
3- **Induce stress in the fish, impacting their immune system and behavior.**

Aim of the project:

The main aim of the project is therefore to identify the effect at the molecular, cellular, anatomical, and physiological levels of ALAN and continuous light regimes.

Project plan and implementation:

Fertilized medaka eggs from different lines (wild type and transgenic lines) will be collected and exposed to continuous light, to ALAN or to 14h light (control group) in the light cabinets (allowing us to control daylength) already available in the model fish facility at the new Veterinary building.

Because medaka larvae are transparent, some live imaging of early brain and heart development will be performed to test the first hypothesis. In addition, after growth, adult fish will be sampled and different organs, including brain and heart, will be collected for morphological analyses neuronal activity by FISH and IF. This work will be done under the supervision of **Dr Beitnes** and **Dr Vindas** who are respectively recognized authorities in the field of fish heart and brain anatomy.

To test the second hypothesis, some adult fish (after puberty), will be sampled. Gonads, brain and pituitary gland, which are key organs in the regulation of the reproductive function, will be collected for investigating differences in gene expression by qPCR and organ anatomy between treatment groups. This will be done at the Fish Neuroendocrine group`s laboratory, headed by **Dr Romain Fontaine**. In our laboratory, we have developed numerous tools, including two medaka transgenic lines where the two pituitary gonadotrope cell types (Lh and Fsh cells) that regulate gametogenesis are respectively labelled with green and red fluorescent markers. These lines will be key tools to identify the cells, allowing us to investigate changes in their activity and number using advance fluorescent imaging (confocal and 3D imaging).

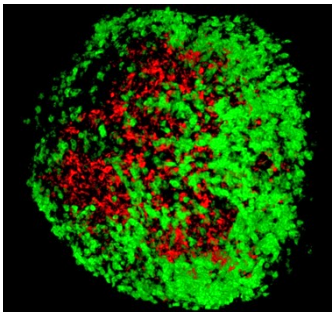

Figure 2: 3D image of the medaka pituitary with the gonadotropes, Lh and Fsh cells respectively labeled with the Green and Red Fluorescent protein (Gfp and Rfp)

Our group just recently published an article demonstrating a novel technique for blood extraction from medaka to measure hormone levels. This will be used to measure cortisol levels in the blood as an

indicator for stress, to test the third hypothesis. In addition, the student will look at the behavior of the animals in adulthood using video recording and tracking softwares to investigate differences between the groups. This will be done under the supervision of **Dr. Ager-Wick** who leads the model fish facility and has recently acquired an advanced tracking system to follow several fish in a tank in the 3 dimensions. Finally, several markers of immune system health monitoring will be measured using protein with multiplex ELISA or gene expression by qPCR analysis with **Dr Lund** who is an expert on the fish immune system.

Materials and methods

Animals: A laboratory fish model - medaka (Orysias Latipes).

Medaka (Oryzias latipes) is a small, egg-laying, freshwater, bony fish that is native to Asian countries (primarily Japan, Korea, and China). Medaka can easily be maintained and bred under laboratory conditions. At sexual maturity, the body length is about 2.5–3 cm, which under proper rearing conditions is achieved within 2 months after hatching. Spawning is under strict control of light, temperature, and food. In adults, on account of their dimorphic dorsal fins, males are easily distinguished from females. In juveniles, determination of the animal gender is also possible because medaka is one of the few teleost fish with genotypic sex determination, thus allowing to investigate sex differences before and during puberty when secondary sexual characters are not yet developed.

Methods:

Quantitative Polymerase Chain Reaction (qPCR): It is a regular laboratory technique of molecular biology which monitors the amplification of a targeted DNA molecule during the PCR (i.e., in real time), not at its end, as in conventional PCR, and thus which provides information on specific gene expression levels in a tissue.

Confocal microscopy: It is an optical imaging technique for increasing optical resolution and contrast of a micrograph using fluorescence. It allows the capture of multiple two-dimensional images at different depths in a sample which enables the reconstruction of three-dimensional structures.

Fluorescent in situ hybridization (FISH) and immune fluorescence (IF): These advanced techniques are used to label in a tissue either mRNA (FISH) or protein (IF) of interest, allowing to investigate cell identity and location as well as changes in cell numbers.

Multiplex ELISA: This technique is a novel type of immunoassay that uses magnetic beads to simultaneously measure multiple analytes in a tissue or blood samples, a single experiment.

Implementation

- The student will be supervised by experts in their field all along the project. He/she will also get support from a young PhD student in the lab, Royan Muhammad Rhamad, and a young master American fellow, Lauren Closs.
- Dr Ida Beitnes, Dr Marco Vindas, Dr Eirill Ager-Wick, Dr Hege Lund and Dr Romain Fontaine are all localized in the new vet building sharing the same lab and office areas, which facilitates communication, particularly in the coordination and supervision of laboratory techniques.

- All the techniques in this project are already established and regularly used in our lab. The fish are raised In the fish facility located in the basement of the new veterinary building allowing easy access for sampling.
- Our group has ample experience with the supervision of students. We have a very international environment which allows for a varied exciting environment were students flourish and thrive.

Subject area (keywords): model fish, physiology, molecular biology, imaging, anatomy, behavior

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Project/company: not part of another project

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